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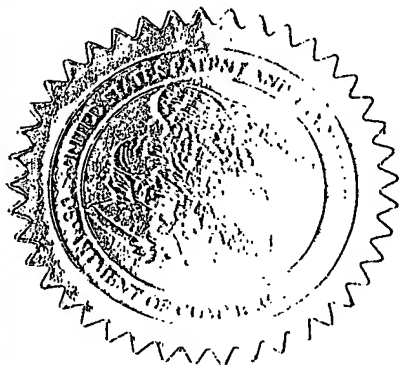
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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TITLE OF THE INVENTION (500 characters max)					
Cyclic Proline-Containing Peptide Mimics					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
<input checked="" type="checkbox"/> Customer Number		23910			
OR Type Customer Number here					
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ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification Number of Pages		4		<input type="checkbox"/> CD(s), Number	
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<input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76					
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT					
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.				FILING FEE AMOUNT (\$)	
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
<input checked="" type="checkbox"/> No.					
<input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: _____					

Respectfully submitted,
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Date March 20, 2003
REGISTRATION NO. 42,349
(if appropriate)
Docket Number: NRNZ-01048US0

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

Attorney Docket No.: NRNZ-1048US0

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Synthesis of Cyclic Proline-Containing Peptide Mimics via Ring-Closing Metathesis

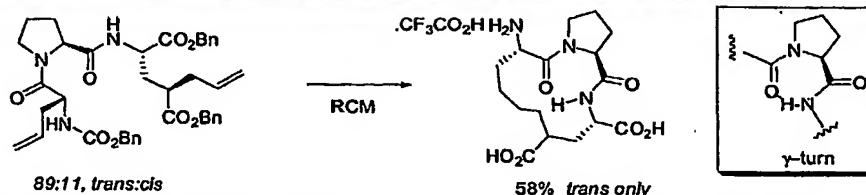
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ABSTRACT



Several dienes embedded in di- and tri-peptides which incorporate proline have been prepared and subjected to ring-closing metathesis. Bicyclic peptide mimics of well-defined amide geometry and of varying ring sizes were prepared. Several limitations of the cyclisation step were revealed.

The use of peptidomimetics¹ to mimic the behavior of biologically active peptides is common in the pursuit of a drug candidate. Peptidomimetics are more metabolically stable and protease resistant than peptides and they often adopt well defined conformations.²

The use of cyclic peptides provides an elegant approach for the synthesis of peptides of rigid geometry that can be used to probe the bioactive conformation of a given peptide. This strategy can be used to establish the structure of a more potent analogue.³

A common method for the synthesis of cyclic peptides⁴ relies on construction of an acyclic precursor, typically a

diene, that then undergoes cyclisation using Grubbs ring-closing metathesis (RCM).⁵ The work reported herein involves the synthesis of dienes from suitable amino acid precursors bearing an allyl group at C(2) or C(5) of proline. RCM of these dienes affords cyclic proline-containing peptides of defined rotamer geometry about the proline amide bond. Several cyclic peptides of varying ring sizes have been prepared and the effect of both stereochemistry and heteroatom location on the metathesis reaction has been investigated.

Synthetic Strategy. We were interested in synthesizing a range of conformationally restricted macrocyclic di- and tri-peptides containing proline, which adopt either *cis* or *trans* stereochemistry about the peptide bond, depending on the substitution on the proline ring.⁶ Given that *cis/trans* isomerase enzymes often deliver the bioactive conformation of a peptide,⁷ peptidomimetics of defined

¹ For recent advances see (a) Aube, J. *Tetrahedron* 2000, 56, 9725-9842; (b) Gante, J. *Angew. Chem., Int. Ed. Engl.* 1994, 33, 1699-1720.

² Giannis, A.; Kolter, T., *Angew. Chem., Int. Ed. Engl.* 1993, 32, 1244-1267.

³ Marshall, G. R. *Tetrahedron*, 1993, 49, 3547-3558.

⁴ (a) Prabhakaran, E. N.; Rao, I. N.; Boruah, A.; Iqbal, J. *J. Org. Chem.* 2002, 67, 8247-8250; (b) Banerji, B.; Bhattacharya, M.; Madhu, R. B.; Das, S. K.; Iqbal, J. *Tetrahedron Lett.* 2002, 43, 6473-6477; (c) Saha, B.; Das, D.; Banerji, B.; Iqbal, J. *Tetrahedron Lett.* 2002, 43, 6467-6471; (d) Banerji, B.; Mallesham, B.; Kumar, S. K.; Kumar, A. C.; Iqbal, J. *Tetrahedron Lett.* 2002, 43, 6479-6483; (e) Belvisi, L.; Colombo, L.; Colombo, M.; Di Giacomo, M.; Manzoni, L.; Vodopivec, B.; Scolastico, C. *Tetrahedron* 2001, 57, 6463-6473; (f) Grossmith, C. E.; Senia, F.; Wagner, J. *Synlett* 1999, 1660-1662; (g) Miller, S. J.; Blackwell, H. E.; Grubbs, R. H. *J. Am. Chem. Soc.* 1996, 118, 9606-9614; (h) Beal, L. M.; Liu, B.; Chu, W.; Moeller, K. D. *Tetrahedron* 2000, 56, 10113-10125; (i) Hoffmann, T.; Lanig, H.; Waibel, R.; Gmeiner, P. *Angew. Chem., Int. Ed.* 2001, 40, 3361-3364.

⁵ (a) Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* 1995, 28, 446-452; (b) Schuster, M.; Blechert, S. *Angew. Chem., Int. Ed. Engl.* 1997, 36, 2037-2056; (c) Armstrong, S. K. *J. Chem. Soc., Perkin Trans. 1* 1998, 371-388; (d) Grubbs, R. H.; Chang, S. B. *Tetrahedron* 1998, 54, 4413-4450; (e) Fürstner, A. *Angew. Chem., Int. Ed. Engl.* 2000, 39, 3012-3043; (f) Trmka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* 2001, 34, 18-29.

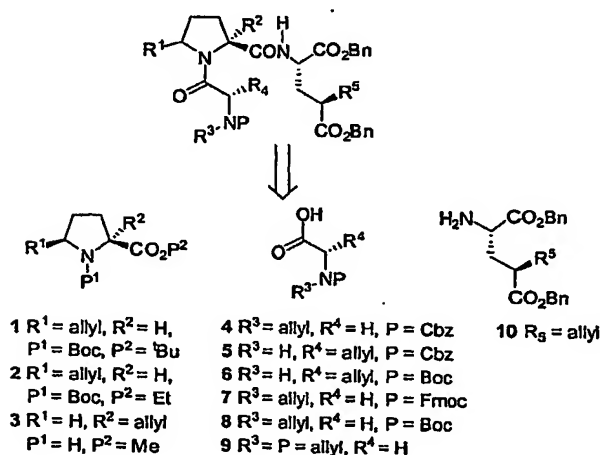
⁶ (a) Halab, L.; Lubell, W. D. *J. Peptide Sci.* 2001, 7, 92-104; (b) Halab, L.; Lubell, W. D. *J. Am. Chem. Soc.* 2002, 124, 2474-2484.

⁷ Fischer, G. *Angew. Chem., Int. Ed. Engl.* 1994, 33, 1415-1436.

conformation are useful for incorporation into a peptide scaffold.

In order to prepare a series of *cis* and *trans* bicyclic dipeptides incorporating proline, it was decided to introduce an allyl group at either the C(2) or C(5) position. We chose a glycylprolylglutamic acid scaffold onto which were also placed an allyl group on either the glycine or glutamate residue (Scheme 1). Introduction of an allyl group onto the α -carbon and the nitrogen atom of glycine was deemed straightforward. Glutamic acid was chosen in view of the well-established methodology for the stereoselective introduction of an allyl group at C(4).⁸

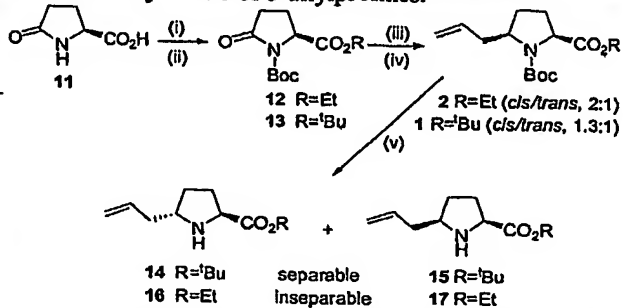
Scheme 1. General synthesis of diene precursors for RCM based on a glycylprolylglutamate scaffold.



Results and Discussion. The synthesis of proline derivatives bearing an allyl group at C(5) has been reported⁹ (Scheme 2). Thus, the ethyl ester¹⁰ and *tert*-butyl ester¹¹ of readily available (*S*)-pyroglutamic acid 11 were protected as their respective Boc carbamates 12¹² and 13.¹³ Selective reduction of the lactam carbonyl with LiEt₃BH yielded aminols, which underwent C-allylation with allyltributylstannane to afford 5-allylprolines 2¹⁴ (*cis/trans*, 66:33) and 1⁹ (*cis/trans*, 57:43) in good yield. Neither set of diastereomers could be separated; however, removal of the Boc group with HCl in dioxane allowed small-scale separation of the *trans* and *cis* *tert*-butyl esters 14 and 15. The stereochemistry of 14 and 15 was determined using nOe experiments and was supported by literature

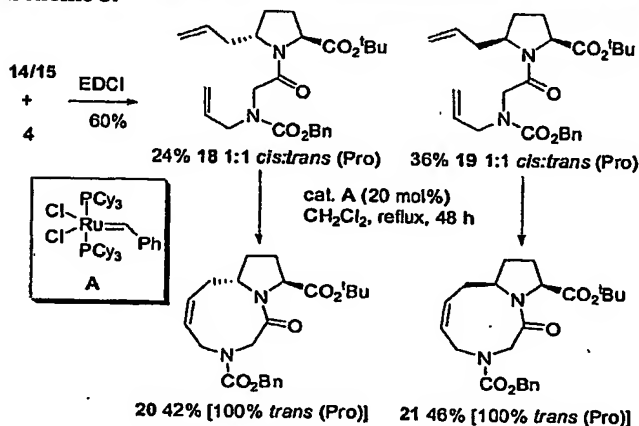
analogues^{9,10} that suggested that the *cis* isomer 15 would be the major product.

Scheme 2. Synthesis of 5-allylprolines.



The preparation of (*S*)-2-allylproline methyl ester 3,¹⁵ *N*-allylglycine carbamates 4,¹⁶ 7,¹⁷ 8,¹⁷ of *C*-allylglycines¹⁸ 5¹⁹ and 6,²⁰ and of glutamate 10⁸ followed literature procedures or variations thereof.

Scheme 3.



Coupling of *N*-allylglycine derivative 4 with the mixture of diastereoisomeric 5-allylprolines 14 and 15 was effected

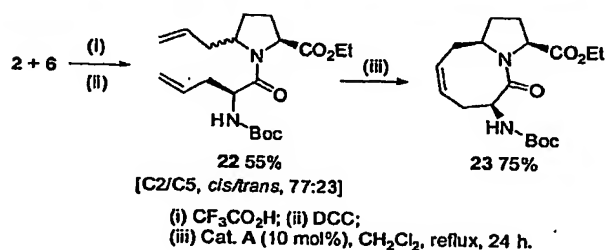
⁸ Hanessian, S.; Margarita, R. *Tetrahedron Lett.* 1998, 39, 5887-5890.
⁹ Chiesa, M. V.; Manzoni, L.; Scolastico, C. *Synlett* 1996, 441-443.
¹⁰ Khim, S.-K.; Cederstrom E.; Ferri, D. C.; Mariano, P. S. *Tetrahedron* 1996, 52, 3195-3222.
¹¹ Besson, M.; Delbecq, F.; Gallezot, P.; Neto, S.; Pinel, C. *Chem. Eur. J.* 2000, 6, 949-958.
¹² Rahul, J. *Org. Prep. Proceed. Int.* 2001, 33, 405-410.
¹³ August, R. A.; Khan, J. A.; Moody, C. M.; Young, D. W. *J. Chem. Soc. Perkin Trans. I* 1996, 6, 506-514.
¹⁴ Mulzer, J.; Schulzchen, F.; Bats, J.-W. *Tetrahedron* 2000, 56, 4289-4298.

¹⁵ (a) Seebach, D.; Boes, M.; Naef, R.; Schweizer, W. B. *J. Am. Chem. Soc.* 1983, 105, 5390-5398; (b) Wang, H.; Germanas, J. P. *Synlett* 1999, 33-36.
¹⁶ Hon, Y.-S.; Chang, R.-C. *Heterocycles* 1991, 32, 1089-1099.
¹⁷ (a) Schleich, S.; Helmchen, G. *Eur. J. Org. Chem.* 1999, 2515-2521; (b) Reichwein, J. F.; Liskamp, R. M. J. *Eur. J. Org. Chem.* 2000, 2335-2344.
¹⁸ (a) Bclokon, Y. N.; Taraov, V. I.; Malcev, V. I.; Savel'eva, T. F.; Ryzhov, M. G. *Tetrahedron: Asymmetry* 1998, 9, 4249-4252; (b) Collet, S.; Bauchat, P.; Danion-Bougot, R.; Danion, D. *Tetrahedron: Asymmetry* 1998, 9, 2121-2131.
¹⁹ Sun, Z.-Y.; Kwon, C.-H.; Wurpel, J. N. D. *J. Med. Chem.* 1994, 37, 2841-2845.
²⁰ Gao, Y.; Lane-Bell, P.; Vederas, J. C. *J. Org. Chem.* 1998, 63, 2133-2143.

with EDCI to yield the separable dienes **18** and **19** in moderate yield (60%). The *cis/trans* ratio of the newly created amide (Pro) bond in both these flexible acyclic dipeptides was 1:1. Ring-closing metathesis of the individual 1,10-dienes **18** and **19** was accomplished using Grubbs catalyst A to give the expected cyclononenes **20** and **21** in moderate yield (Scheme 3). The ring closure was not affected by the stereochemistry of the allyl group at C(5) on the proline moiety, both reactions giving similar yields with comparable reaction times and catalyst loadings. Very little deallylation/isomerisation occurred (*cf.* *N*- or *O*-allyl systems).²¹ In contrast to their respective precursors, both macrocycles **20** and **21** adopted only the *trans* conformation about the peptide bond.

Subsequent transformations of **20** and **21** proved to be problematic due to the presence of the *tert*-butyl ester and a benzylcarbamate hence our attention was turned to the use of an ethyl ester with a Boc as protecting group. Due to difficulties in separating the ethyl esters **16** and **17**, the mixture of diastereomers **2** was coupled with the C(2)-allylglycine **6** using DCC to afford an inseparable mixture of dipeptides **22** [C(2)/C(5), *cis/trans*, 77:23, *cis/trans* (Pro) ratio undetermined due to complex NMR spectra] in 55% yield (Scheme 4). Subjecting the diene mixture to the standard RCM conditions yielded only one macrocycle **23** in excellent yield (75%). Based on the fact that the *cis* diastereomer was the major component in the precursor, the cyclic metathesis product was assigned as *cis* C(2)/C(5) stereochemistry. Clearly the minor *trans* isomer cyclises extremely slowly relative to the *cis* isomer, a trend that was not observed earlier in the metathesis of the *N*-allyl dienes **18** and **19** (Scheme 3). Gratifyingly cyclooctene **23** adopted only *trans* stereochemistry about the peptide bond.

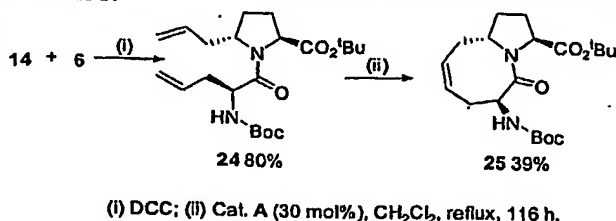
Scheme 4.



In order to access the C(2)/C(5) *trans* diastereomer **25** of **23** it was necessary to use amine **14**; coupling with C(2)-allylglycine **6** using DCC yielded the diene **24** in 80% yield [Scheme 5, *cis:trans* (Pro) ratio undetermined due to complex NMR spectra]. As anticipated ring closure of **24** was extremely slow, required high catalyst loading, and afforded a lower yield of the cyclooctene **25**. However, the

peptide bond within macrocycle **25** adopted only a *trans* conformation.

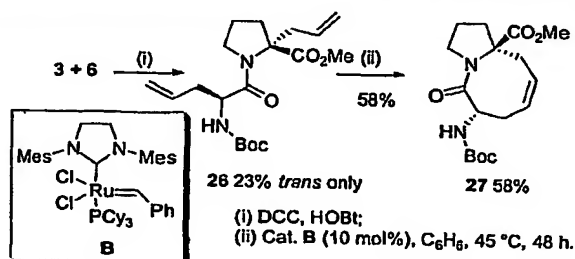
Scheme 5.



Grubbs *et al.*²² have reported that certain *cis* substituted cyclohexane derivatives metathesise under more forcing conditions and in lower yield than the corresponding *trans* isomers. In the present work it was observed that placing the allyl moieties closer together (*C*-allyl vs *N*-allyl) and in certain orientations (*cis* vs *trans*) resulted in significant differences in reactivity.

The above metathesis reactions had involved an allyl group at C(5) on the proline and an allyl unit on the glycine, forming macrocycles containing a *trans* (Pro) amide bond. In order to obtain macrocycles with a *cis* (Pro) amide bond, metathesis between an allyl group at C(2) on proline and an allyl group on the glycine was required. Thus metathesis of **26** with the second generation Grubbs's catalyst (**B**)²³ yielded bicyclic cyclooctene **27**th as a single (*cis*) rotamer in good yield (Scheme 6).

Scheme 6.



All attempts to metathesise the corresponding C(2) allyl/*N*-allyl derivatives **28**, **29**, or **30** (Scheme 7) gave complex mixtures probably due to competing deallylation, which is accelerated by the electron-withdrawing Boc, CO₂Bn or Fmoc carbamates. *N,N'*-Diallylglycine²⁴ derivative **31** was prepared in an attempt to avoid this effect; however, only starting material was recovered after

²¹ (a) Cadot, C.; Dalko, P. I.; Cossy, J. *Tetrahedron Lett.* 2002, 43, 1839-1841; (b) Alcaide, B.; Almendros, P.; Alonso, J. M.; Aly, F. M. *Org. Lett.* 2001, 3, 3781-3784.

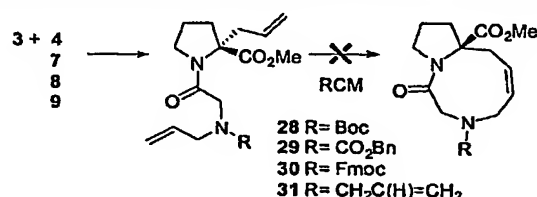
²² Miller, S. J.; Kim, S.-H.; Chen, Z.-R.; Grubbs, R. H. *J. Am. Chem. Soc.* 1995, 117, 2108-2109.

²³ Morgan, J. P.; Grubbs, R. H. *Org. Lett.* 2000, 2, 3153-3155.

²⁴ (a) Garro-Helion, F.; Merzouk, A.; Guibe, F. *J. Org. Chem.* 1993, 58, 6109-6113; (b) Hassner, A.; Maurya, R.; Friedman, O.; Gottlieb, H. E.; Padwa, A.; Austin, D. *J. Org. Chem.* 1993, 58, 4539-4546.

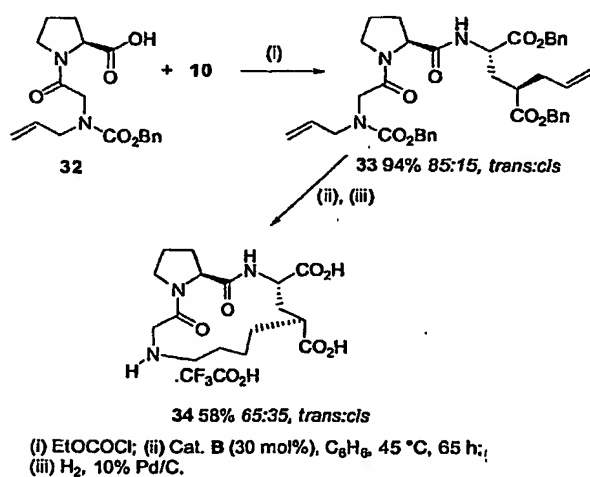
attempted RCM, possibly due to quenching of the catalyst by the reaction with the more basic nitrogen.²⁵ Conversion of diallylamine **31** into its hydrochloride salt²⁶ followed by attempted RCM was also unsuccessful.

Scheme 7.



The ring-closing metathesis of dienes contained within a Gly.Pro.Glu scaffold was also examined. The substrate was synthesized (Scheme 8) from coupling **32** with **10** to yield *N*-allylglycyl diene **33**, predominantly as the *trans* (Pro) rotamer. Exposure of **33** to Grubbs catalyst **B** followed by hydrogenation gave macrocycle **34** in 58% yield after purification by HPLC. Cyclotetradecene **34** existed as a 65:35 mixture of *trans:cis* (Pro) rotamers. The increased proportion of the *cis* rotamer may reflect increased flexibility of the Pro amide bond when it is embedded in a larger 14 membered ring.

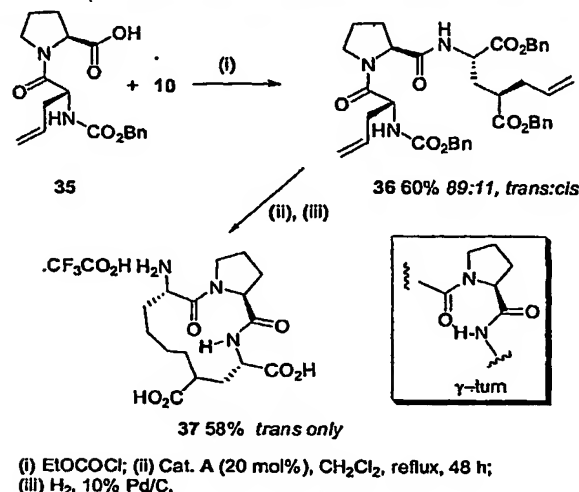
Scheme 8.



Metathesis of the corresponding *C*-allylglycyl dipeptide **36** and subsequent global deprotection yielded the 13-membered macrocycle **37** (58%) as a single *trans* rotamer (Scheme 9). The observation of only the *trans* amide

rotamer may be a consequence of the smaller ring size and/or stabilisation of the structure by the formation of a γ -turn.

Scheme 9.



Summary. The synthesis and ring closure of a number of di- and tri-peptides incorporating proline has been accomplished via construction of appropriate diallylated peptide precursors and subsequent olefin metathesis. The proline containing peptides cyclised to afford macrocycles that adopted (except for **34**) a single conformation about the Gly-Pro amide bond. The conformation adopted in each case however was dependant on both the point of attachment of the diene (*C*-allyl vs *N*-allyl) and on the *cis-trans* stereochemistry [C(2) vs C(5)] on the proline ring.

Acknowledgment. The authors thank NeuronZ Ltd for financial support.

Supporting Information Available: Experimental procedures and spectral data for compounds **20**, **23**, and **37**.

²⁵ Shon, Y.-S.; Lee, T. R. *Tetrahedron Lett.* 1997, 38, 1283-1286.

²⁶ Fu, G. C.; Nguyen, S. T.; Grubbs, R. H. *J. Am. Chem. Soc.* 1993, 115, 9856-9857.